

Diatom cleaning with nitric/sulfuric acids

PREAMBLE

Different methods can be used to oxidize the organic material of diatoms and to clean the siliceous components of the frustule. The use of acids is recommended for well silicified species.

GENERAL RECOMMENDATION

Carefully follow safety procedures when working with acids.

1. Centrifuge 10 ml of concentrated sample or culture in a glass centrifuge tube at 3000-3500 rpm (revolutions per minute) for at least 10 minutes.
2. Remove the supernatant with a pipette, without disturbing the pellet on the bottom of the tube.
3. Resuspend in distilled water and repeat centrifugation and washing at least 3 times to eliminate salt.
4. Add to the pellet, under fume hood, one volume of 65% HNO₃ and 4 volumes of 98% H₂SO₄ (1:1:4, sample: HNO₃: H₂SO₄).
5. Heat the mixture over a flame holding the tubes with tweezers and let it boil for few seconds; repeat the procedure.
6. Let it cool down for 30 minutes.
7. Rinse several times with distilled water and centrifuge to remove any residual acid.
8. Check that pH is neutral with a paper indicator.

Note: For delicate species, centrifugation might be substituted by passive sedimentation.

Cleaned diatom material can be used for:

1. TEM observation
2. SEM observation
3. preparation of permanent slides

Cleaned diatom material can be stored in distilled water with few drops of formaldehyde and acetic acid to avoid growth of fungi and bacteria and the dissolution of silica.

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Algae: fixed or alive, natural or culture
sample containing diatoms

Apparatus:

fume hood, centrifuge, Bunsen burner,
Glass centrifuge tubes, tweezers

Solutions: nitric acid (HNO₃), sulfuric acid
(H₂SO₄), distilled water.

Plasticware: disposable pipettes

Additional information:

Hasle, G. R. (1978) - *Diatoms. In Sournia
A. (Ed.), Phytoplankton Manual.
UNESCO Monograph on Oceanography
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Round, F.E., Crawford, R.M. & Mann,
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University Press, Cambridge. 747.*

ASSEMBLE

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